Figure 1

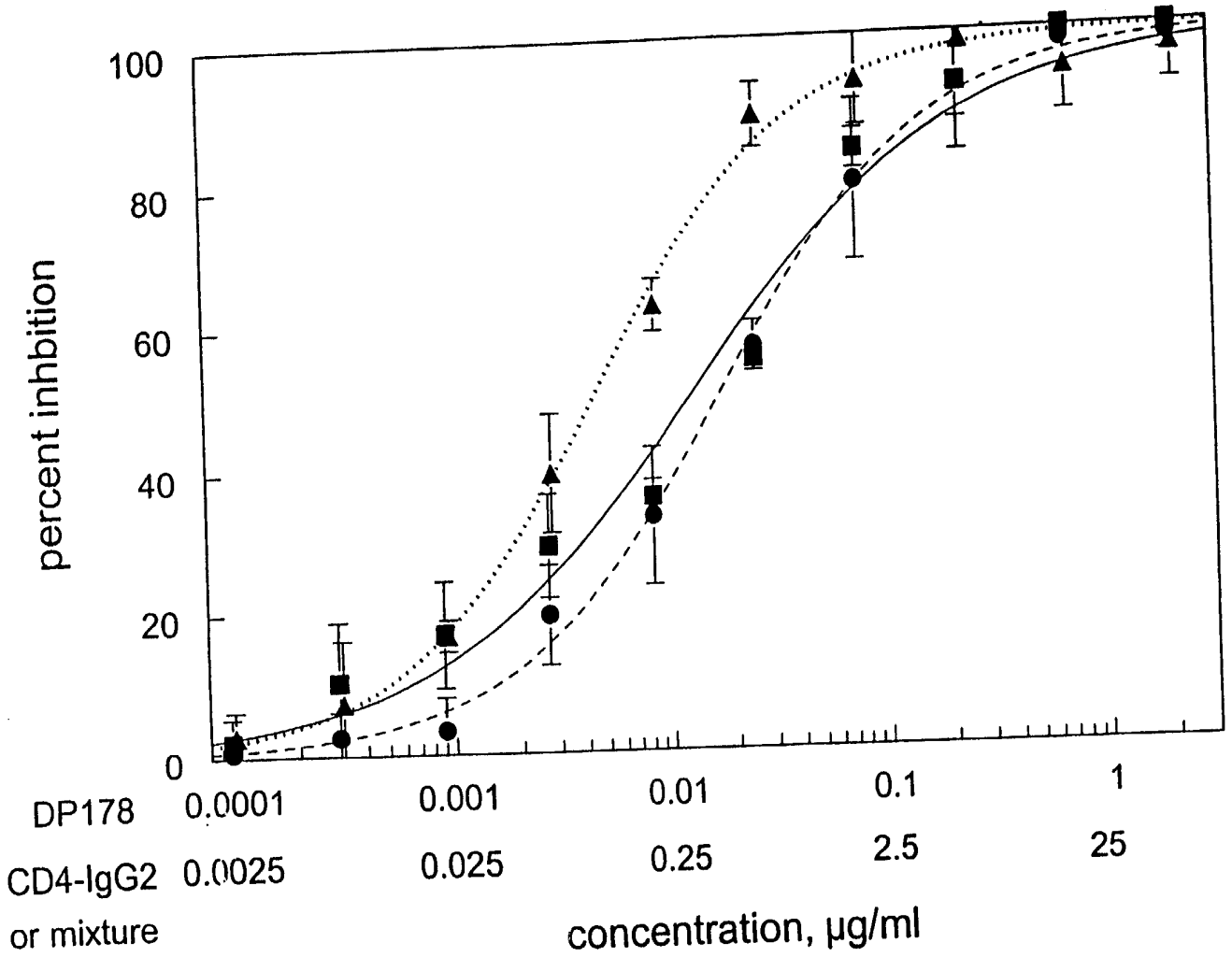
100
80
60
40
20
0
percent inhbition
DP178
CD4-IgG2
or mixture
0.0001
0.0025
0.001
0.025
0.01
0.25
0.1
2.5
1
25
concentration, µg/ml

Figure 2

Percent Inhibition	Combination Index CD4-IgG2:T-20 Mass Ratio 25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 3

Percent Inhibition	T-20 Concentration, μg/ml Alone	T-20 Concentration, μg/ml Combination	T-20 Dose Reduction	CD4-IgG2 Concentration, μg/ml Alone	CD4-IgG2 Concentration, μg/ml Combination	CD4-IgG2 Dose Reduction
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 4A

Percent Inhibition	Combination Index	PRO 542 Concentration, nM Alone	PRO 542 Concentration, nM Mix	PRO 542 Dose Reduction	PA12 Concentration, nM Alone	PA12 Concentration, nM Mix	PA12 Dose Reduction	T-20 Concentration, nM Alone	T-20 Concentration, nM Mix	T-20 Dose Reduction
95	0.41	10	2.1	4.8	730	2.8	260	94	19	4.9
90	0.45	7.0	1.6	4.4	320	2.1	150	63	14	4.5
70	0.47	4.1	0.92	4.5	72	1.2	60	30	8.1	3.7
50	0.48	3.1	0.66	4.7	28	0.87	32	19	5.8	3.3

PRO 542, PA12 and T-20 were used in an approximate 1:1:10 molar concentration ratio.

Figure 4B

Percent Inhibition	Combination Index	PRO 542 Concentration, nM Alone	PRO 542 Concentration, nM Mix	PRO 542 Dose Reduction	PRO 140 Concentration, nM Alone	PRO 140 Concentration, nM Mix	PRO 140 Dose Reduction	T-20 Concentration, nM Alone	T-20 Concentration, nM Mix	T-20 Dose Reduction
95	0.40	8.5	1.9	4.5	19	1.0	19	140	17	8.2
90	0.39	7.1	1.5	4.7	13	0.77	17	100	13	7.7
70	0.37	5.3	0.87	6.1	7.2	0.46	16	57	7.7	7.4
50	0.35	4.6	0.63	7.3	4.9	0.34	14	40	5.6	7.1

PRC 542, PRO 140 and T-20 were used in an approximate 2:1:20 molar concentration ratio.

Figure 4C

Percent Inhibition	Combination Index	PRO 542 Concentration, nM Alone	PRO 542 Concentration, nM Mix	PRO 542 Dose Reduction	PRO 140 Concentration, nM Alone	PRO 140 Concentration, nM Mix	PRO 140 Dose Reduction	T-20 Concentration, nM Alone	T-20 Concentration, nM Mix	T-20 Dose Reduction
95	0.24	61	2.5	24	11.9	0.72	17	156	22	7.1
90	0.22	32	1.4	23	8.4	0.40	21	96	13	7.4
70	0.19	9.8	0.50	20	4.5	0.14	32	40	4.5	8.9
50	0.18	4.7	0.26	18	3.0	0.074	41	23	2.3	10

PRO 542, PRO 140 and T-20 were used in an approximate 4:1:30 molar concentration ratio.

Figure 4D

Percent Inhibition	Combination Index	PRO 140 Concentration, nM Alone	PRO 140 Concentration, nM Mix	PRO 140 Dose Reduction	T-20 Concentration, nM Alone	T-20 Concentration, nM Mix	T-20 Dose Reduction
95	0.56	12	1.8	6.7	156	55	2.8
90	0.55	8.4	1.1	7.4	96	35	2.7
70	0.55	4.5	0.51	8.8	40	16	2.5
50	0.56	3.0	0.31	9.9	23	10	2.4

PRO 140 and T-20 were used in an approximate 1:30 molar concentration ratio.

Figure 5

Triple Combination Synergistically Blocks HIV-1 Entry (I)

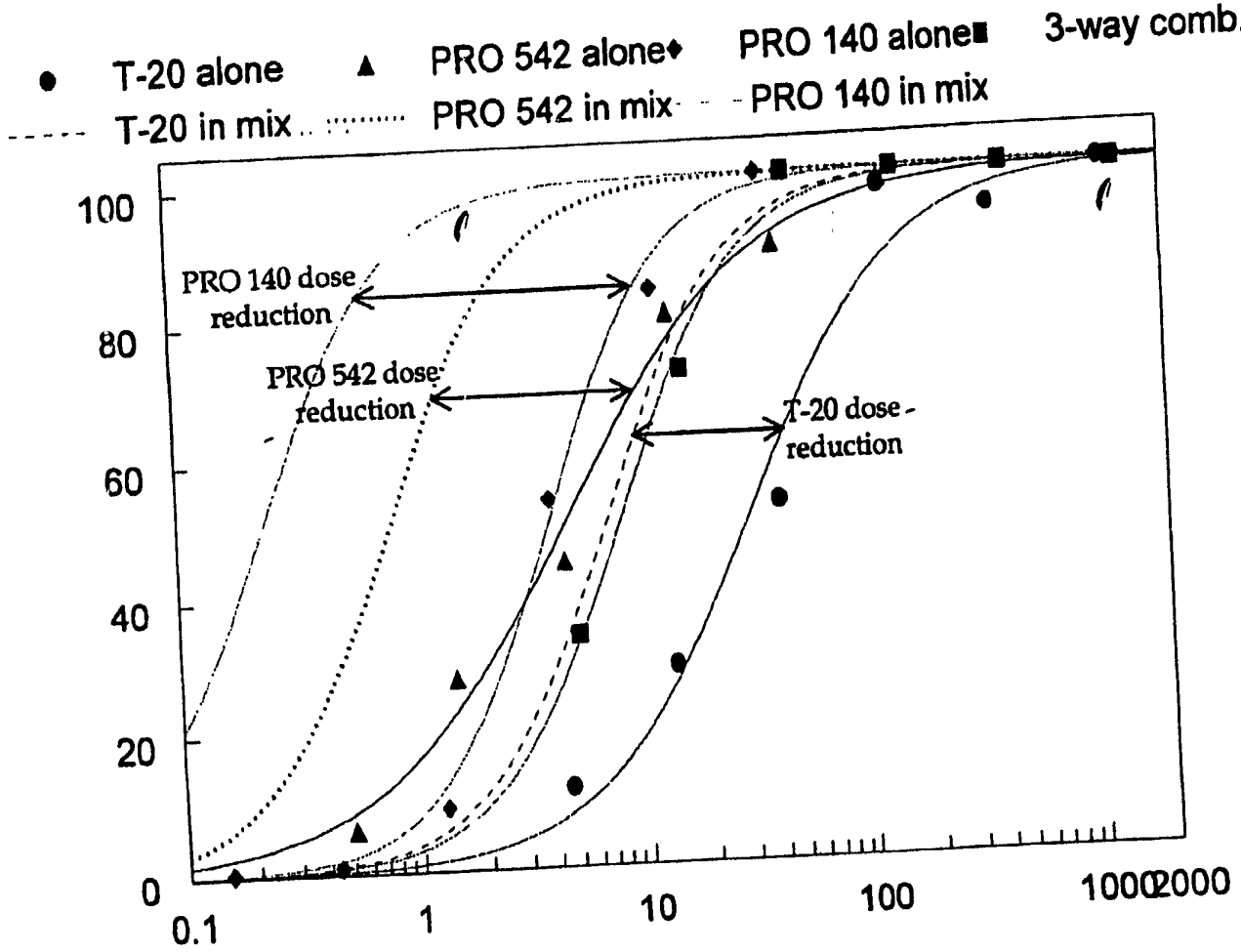

Figure 6

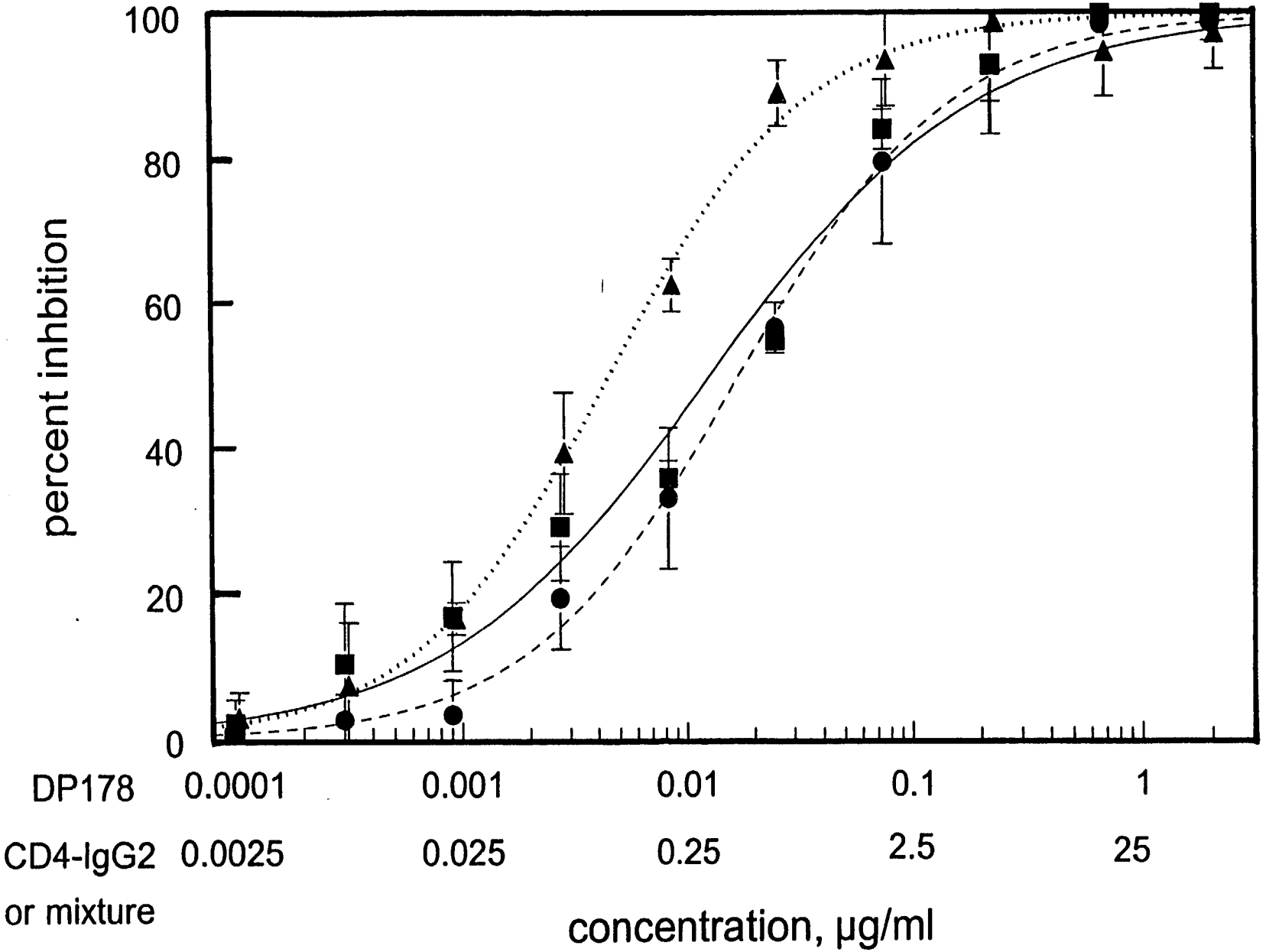
100
80
60
40
20
0
percent inhbition
DP178 0.0001 0.001 0.01 0.1 1
CD4-IgG2 0.0025 0.025 0.25 2.5 25
or mixture
concentration, µg/ml

Figure 7

	Combination Index			
	CD4-IgG2:T-20 Mass Ratio			
Percent Inhibition	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 8

Percent Inhibition	T-20 Concentration, μg/ml		T-20 Dose Reduction	CD4-IgG2 Concentration, μg/ml		CD4-IgG2 Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 9

Assay (virus)	PRO 542:T-20 Molar Ratio	Percent Inhibition	Combination Index	PRO 542			T-20		
				Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
				Alone	Mix		Alone	Mix	
Virus-cell fusion (JR-FL)	1:2	95	0.14	30	2.8	11	120	5.1	24
		90	0.18	12	1.5	8.0	45	2.6	17
		70	0.29	2.5	0.44	5.7	8.0	0.78	10
		50	0.39	0.92	0.21	4.4	2.7	0.37	7.3
Virus-cell fusion (DH123)	1:2	95	0.36	65	11	5.9	123	20	6.2
		90	0.45	20	5.0	4.0	54	8.9	6.1
		70	0.76	2.4	1.2	2.0	12	2.1	5.7
		50	1.1	0.64	0.49	1.3	4.8	0.87	5.5
Cell-cell fusion (JR-FL)	1:2	95	0.36	35	6.3	5.6	73	11	6.6
		90	0.43	14	3.2	4.4	34	5.8	5.9
		70	0.61	2.9	0.94	3.1	8.5	1.7	5.0
		50	0.76	1.0	0.43	2.3	3.6	0.78	4.6
Cell-cell fusion (JR-FL)	1:10	95	0.27	28	1.4	20	58	12	4.8
		90	0.28	11	0.55	20	22	4.9	4.5
		70	0.31	2.3	0.11	21	3.8	0.97	3.9
		50	0.34	0.84	0.039	17	1.3	0.35	3.7
Cell-cell fusion (JR-FL)	1:50	95	0.33	47	0.84	56	120	37	3.2
		90	0.34	15	0.30	50	42	13	3.2
		70	0.36	1.8	0.045	40	6.1	2.0	3.0
		50	0.38	0.49	0.014	35	1.8	0.61	3.0

Figure 10

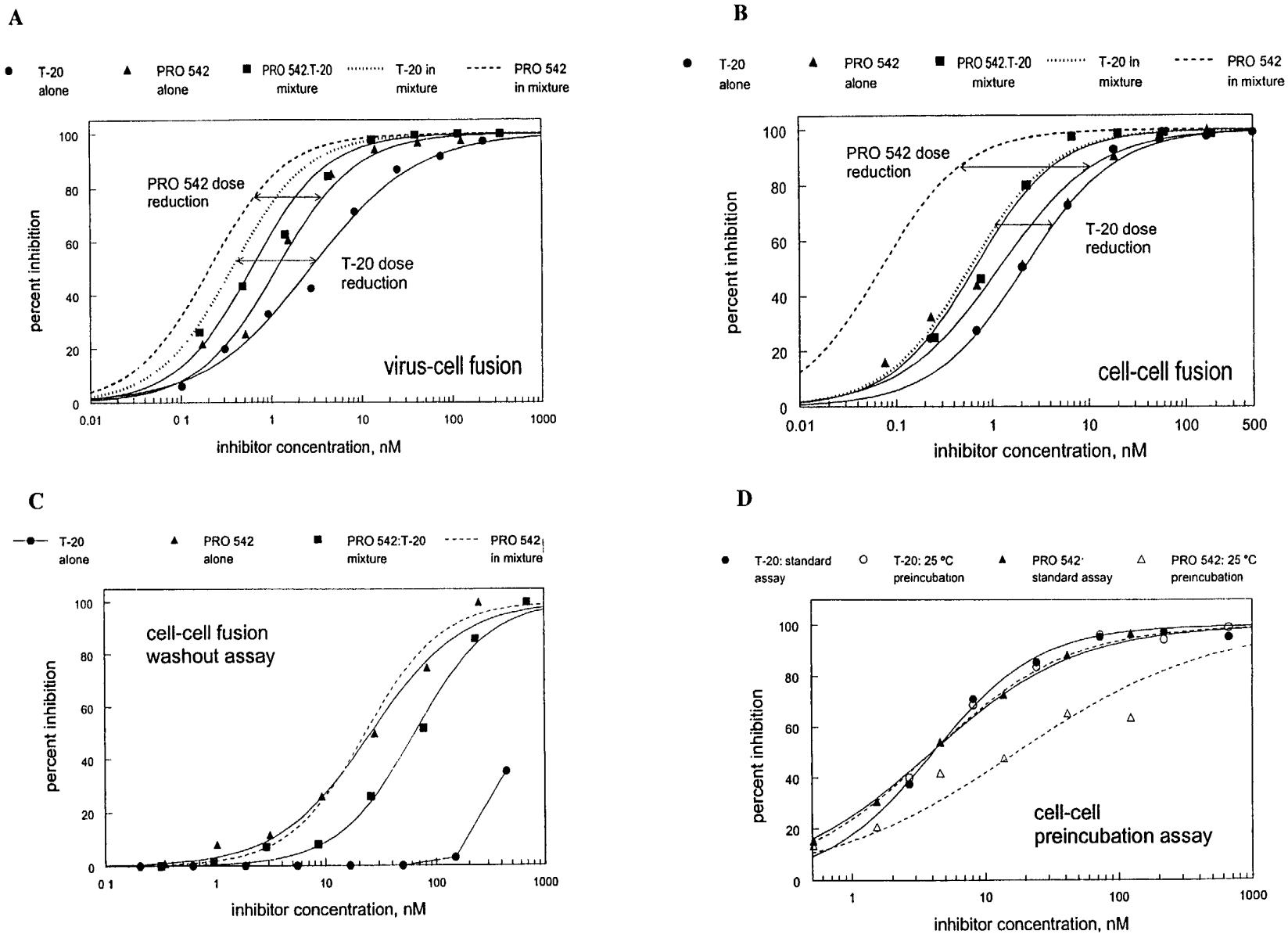
A
T-20 alone
PRO 542 alone
PRO 542.T-20 mixture
T-20 in mixture
PRO 542 in mixture
PRO 542 dose reduction
T-20 dose reduction
virus-cell fusion
percent inhibition
inhibitor concentration, nM
B
T-20 alone
PRO 542 alone
PRO 542.T-20 mixture
T-20 in mixture
PRO 542 in mixture
PRO 542 dose reduction
T-20 dose reduction
cell-cell fusion
percent inhibition
inhibitor concentration, nM
C
T-20 alone
PRO 542 alone
PRO 542:T-20 mixture
PRO 542 in mixture
cell-cell fusion washout assay
percent inhibition
inhibitor concentration, nM
D
T-20: standard assay
T-20: 25 °C preincubation
PRO 542: standard assay
PRO 542: 25 °C preincubation
cell-cell preincubation assay
percent inhibition
inhibitor concentration, nM

Figure 11

HIV-1 Entry Involves at Least Three Steps that Provide Promising Targets for Therapy

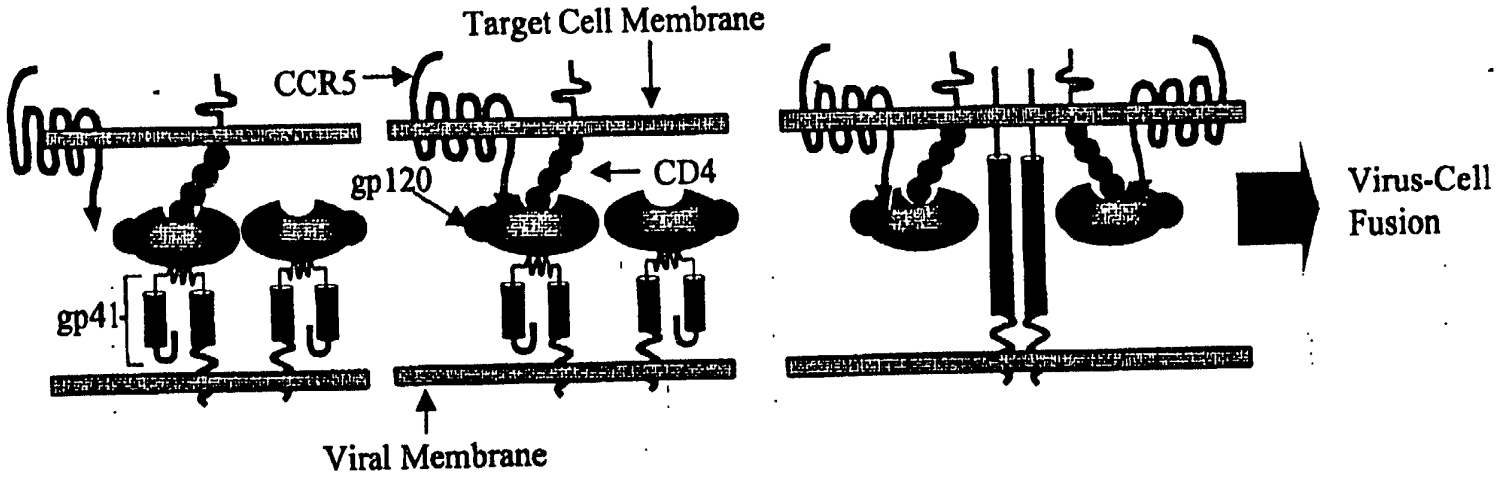

Figure 12

PRO 542 (CD4-IgG2)
attachment inhibitor

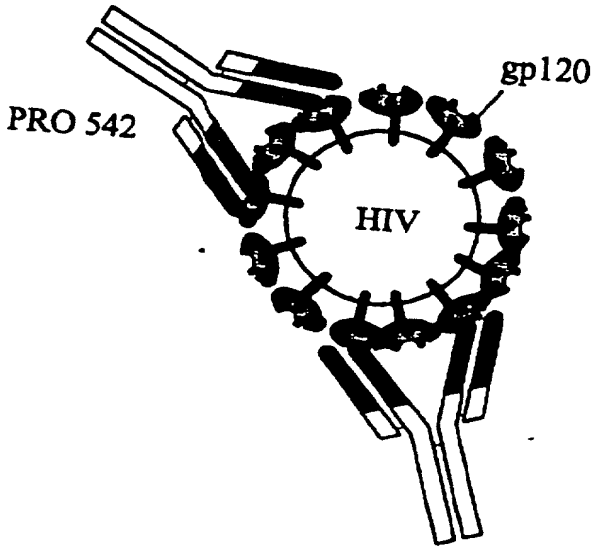

Figure 13

PRO 140

coreceptor inhibitor

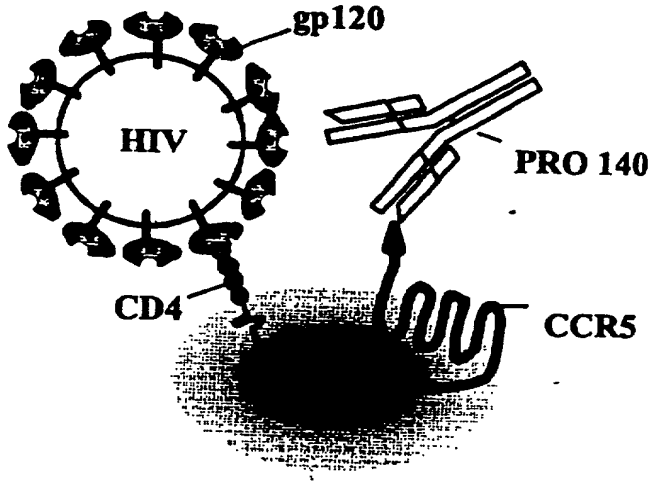

Figure 14

T-20
fusion inhibitor

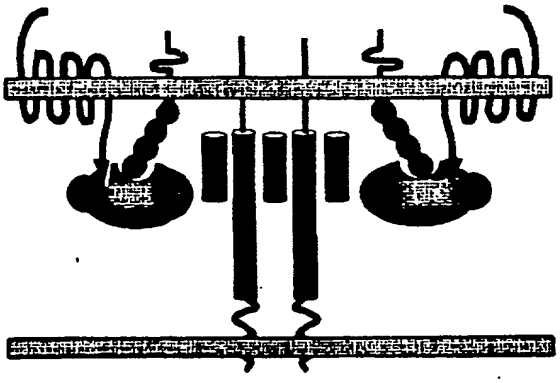

Figure 15

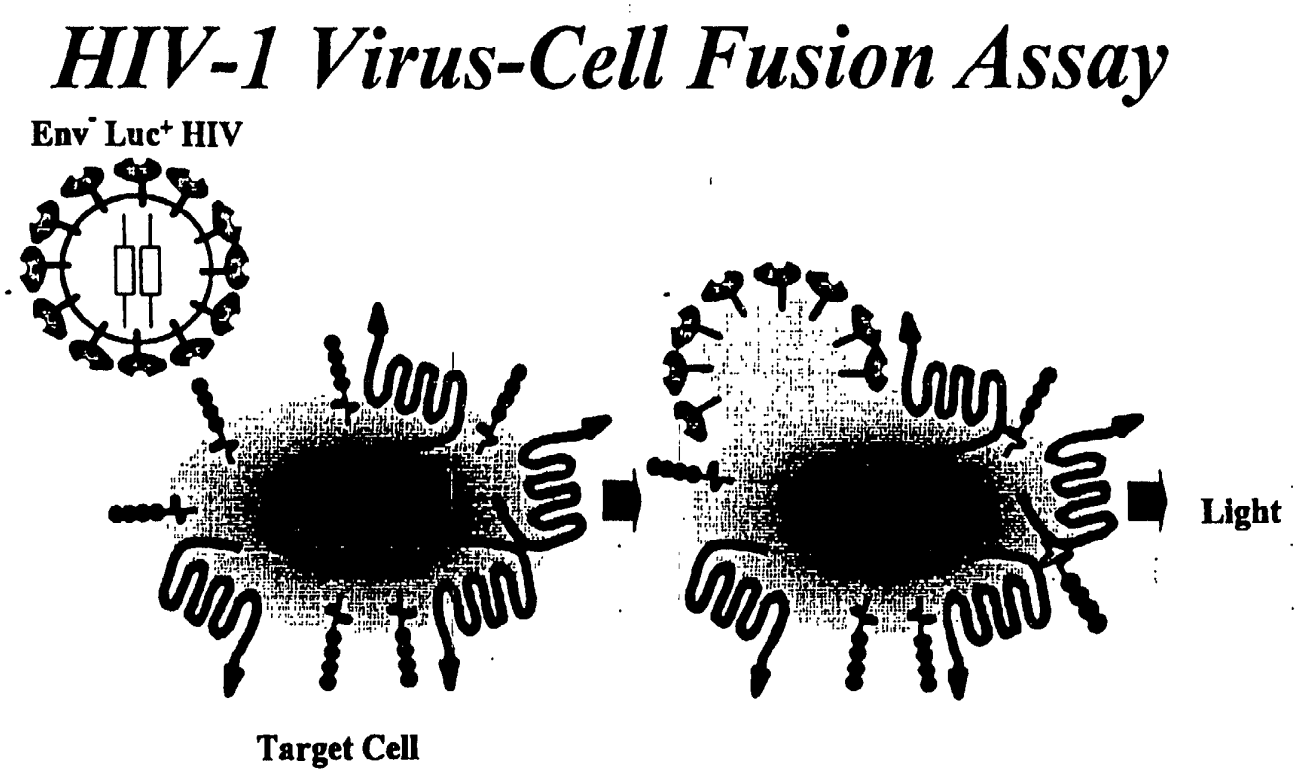

Figure 16

Synergistic Inhibition of Virus-Cell Fusion with PRO 542 and T-20 (I)

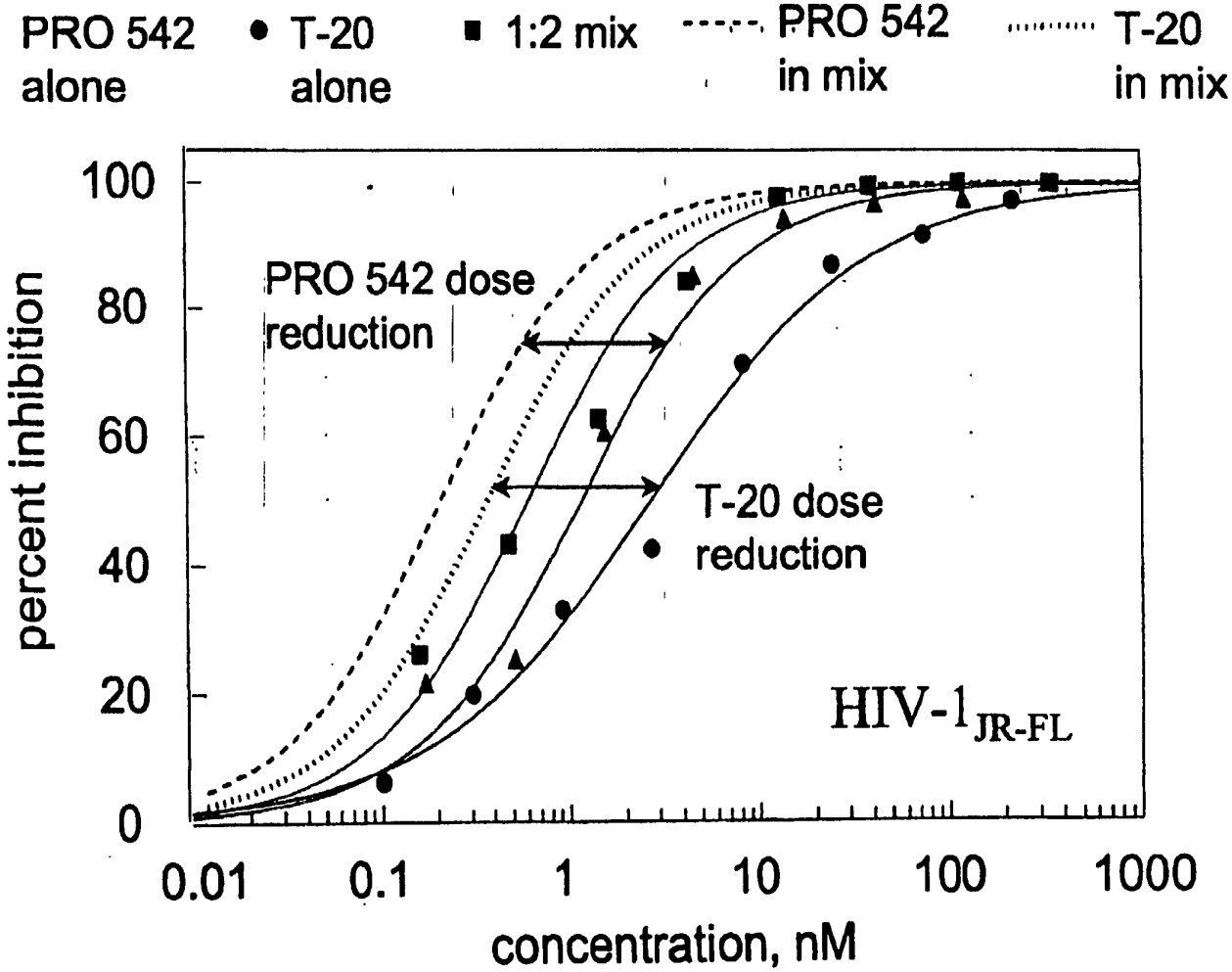

Figure 17

Synergistic Inhibition of HIV-1 Virus-Cell Fusion with PRO 542 and T-20 (II)

	Percent Inhibition	Combination Index	Inhibitory Conc., nM		Dose Reduction	
			PRO 542	T-20	PRO 542	T-20
JR-FL (R5)	95	0.14	30	120	11	24
	90	0.18	12	45	8.0	17
	70	0.29	2.5	8.0	5.7	10
	50	0.39	0.92	2.7	4.4	7.3
DH123 (R5X4)	95	0.36	65	123	5.9	6.2
	90	0.45	20	54	4.0	6.1
	70	0.76	2.4	12	2.0	5.7
	50	1.1	0.64	4.8	1.3	5.5

PRO 542 and T-20 were used in a 1:2 molar ratio

Figure 18

HIV-1 Cell-Cell Fusion Assay

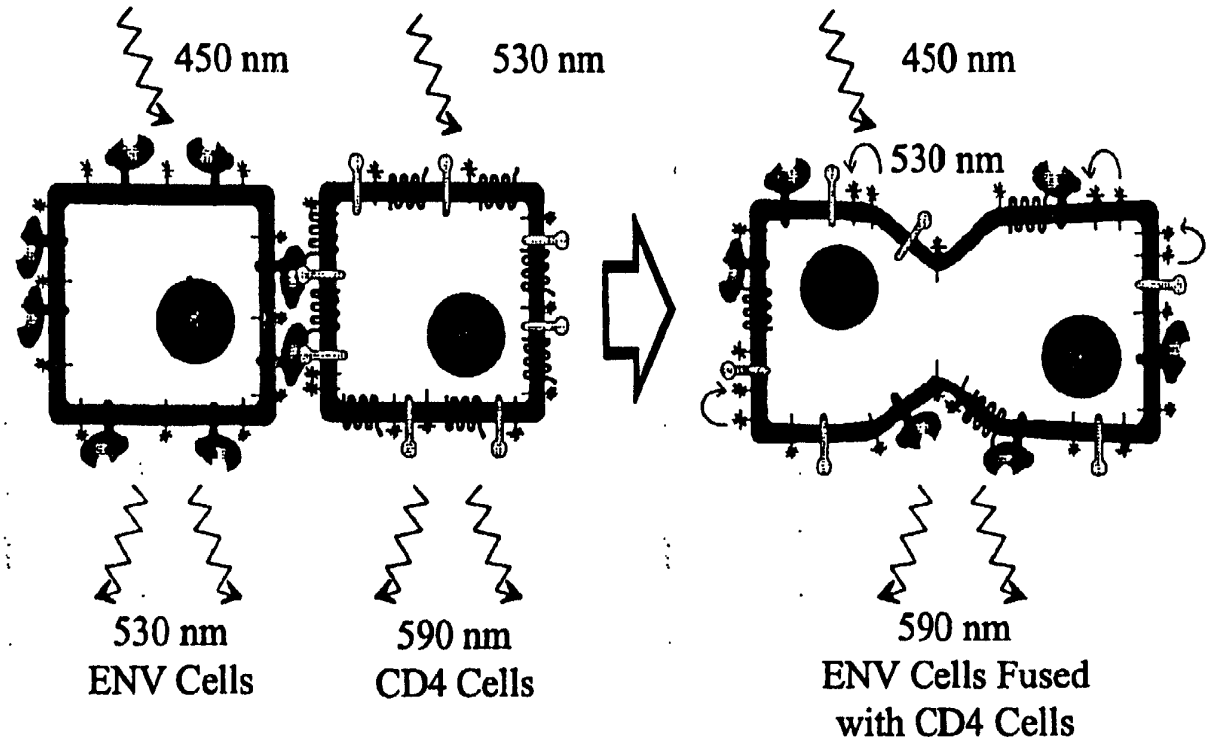

Figure 19

Synergistic Inhibition of Cell-Cell Fusion with PRO 542 and T-20 (I)

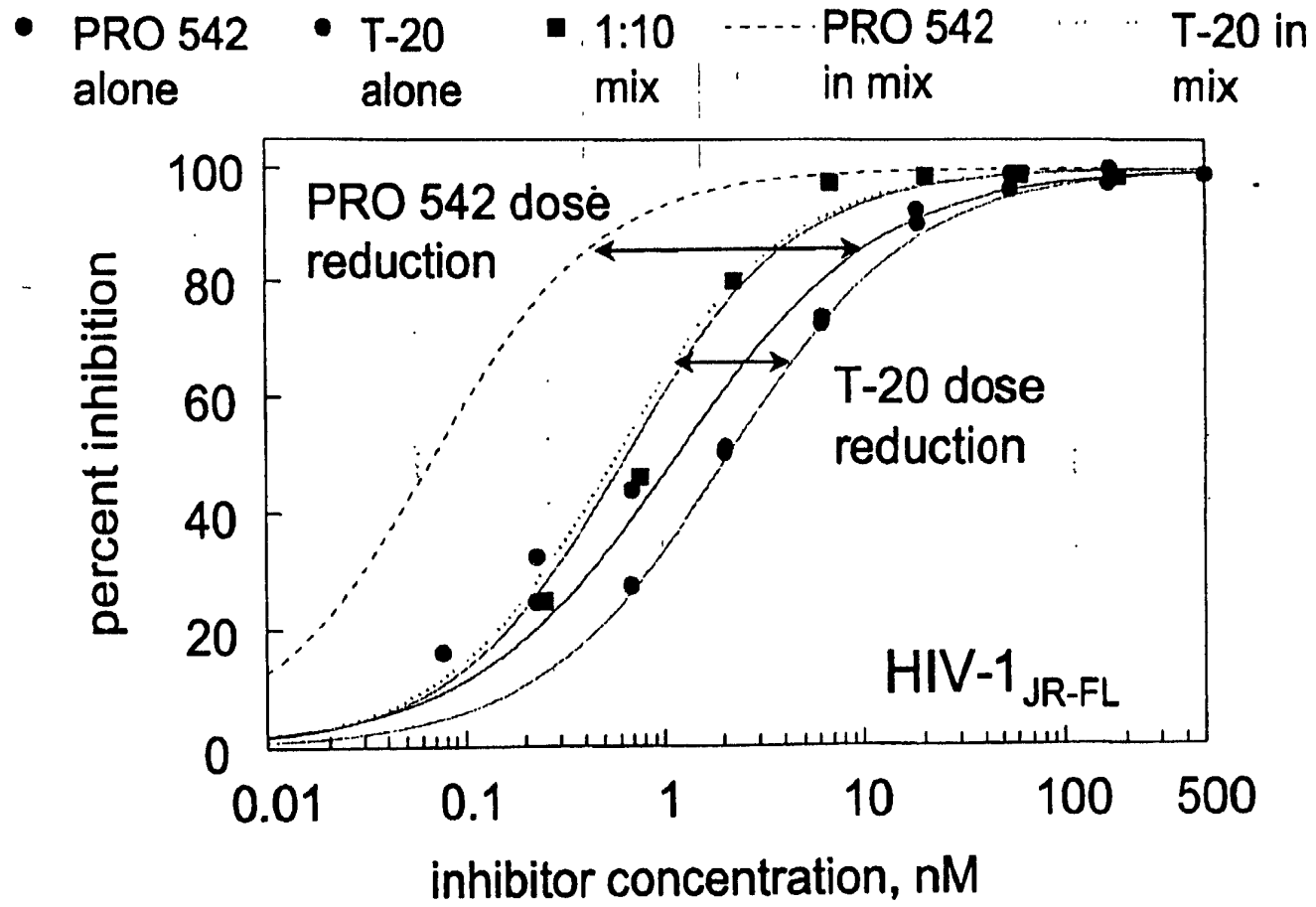

Figure 20

Synergistic Inhibition of HIV-1 Cell-Cell Fusion with PRO 542 and T-20 (II)

Conc. Ratio	Percent Inhibition	Combination Index	Inhibitory Conc, nM		Dose Reduction (fold)	
			PRO 542	T-20	PRO 542	T-20
1:2	95	0.32	95	47	17	4.9
	90	0.38	39	22	13	4.2
	50	0.69	3.0	2.5	6.2	2.8
1:10	95	0.27	28	58	20	4.8
	90	0.28	11	22	20	4.5
	50	0.34	0.84	1.3	22	3.7
1:50	95	0.33	47	120	56	3.2
	90	0.34	15	42	50	3.2
	50	0.38	0.49	1.8	35	3.0

Virus: $HIV\text{-}1_{JR\text{-}FL}$

Figure 21

PRO 140, PRO 542 and T-20 Triple Combination Synergistically Blocks HIV-1 Entry (I)

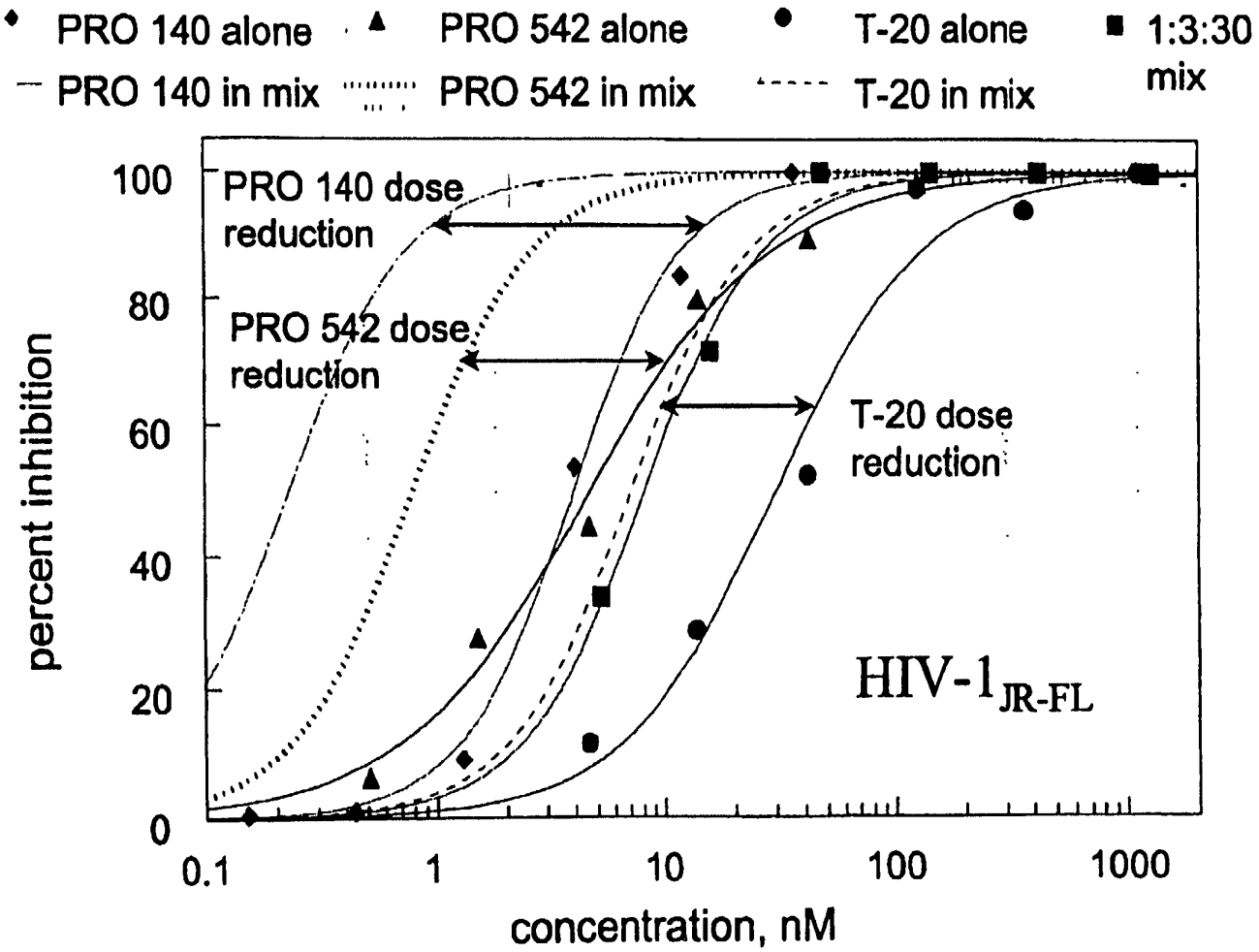

Figure 22

PRO 140, PRO 542, T-20 Triple Combination Synergistically Blocks HIV-1 Entry (II)

Percent Inhibition	Combination Index	Inhibitory Conc, nM			Dose Reduction (fold)		
		PRO 140	PRO 542	T-20	PRO 140	PRO 542	T-20
95	0.24	24	61	160	17	12	7.1
90	0.22	23	32	96	21	8.4	7.4
70	0.19	20	9.8	40	32	4.5	8.9
50	0.18	18	4.7	23	41	3.0	10

Inhibition of HIV-1_{JR-FL} mediated cell-cell fusion with PRO 140, PRO 542 and T-20 used in a 1:3:30 molar ratio.

Figure 23

PRO 542 Does Not Potentiate T-20 Activity in the Absence of Coreceptor

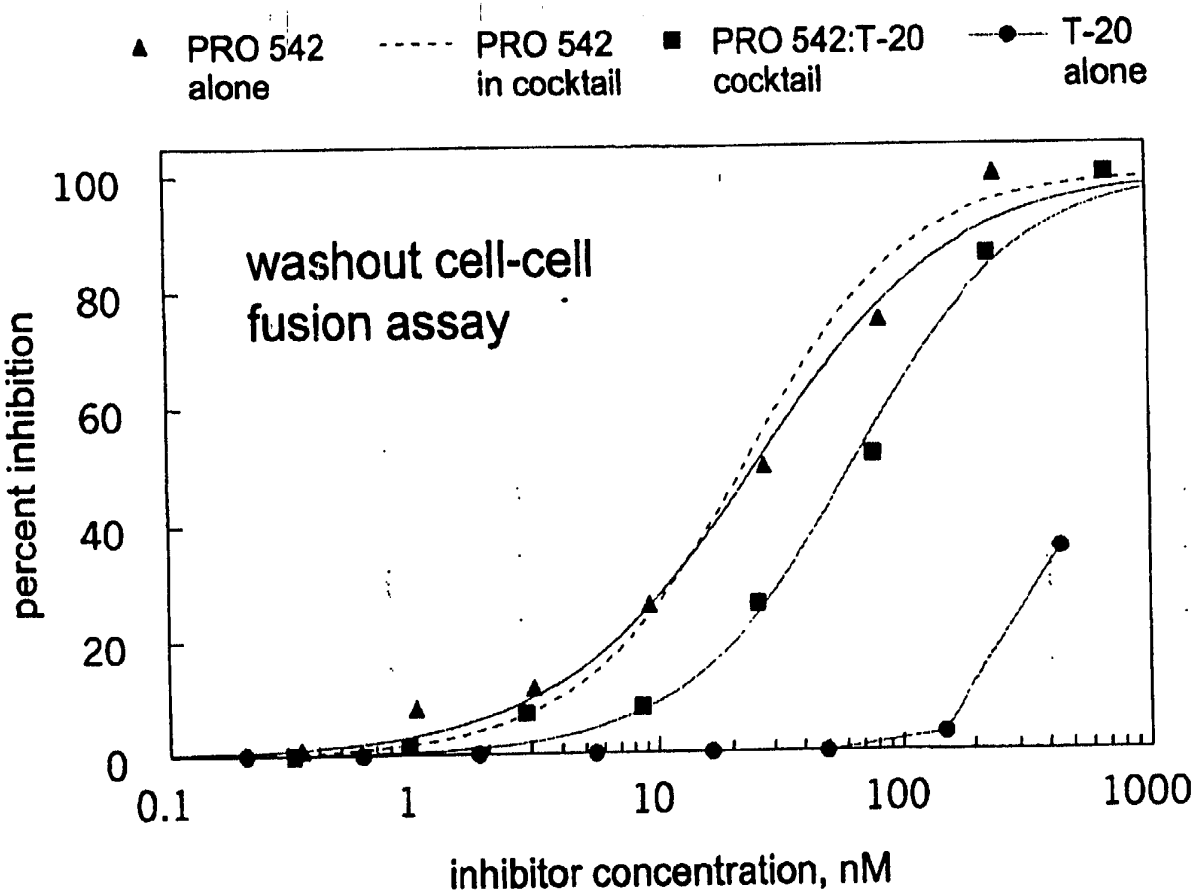

Figure 24

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (I)

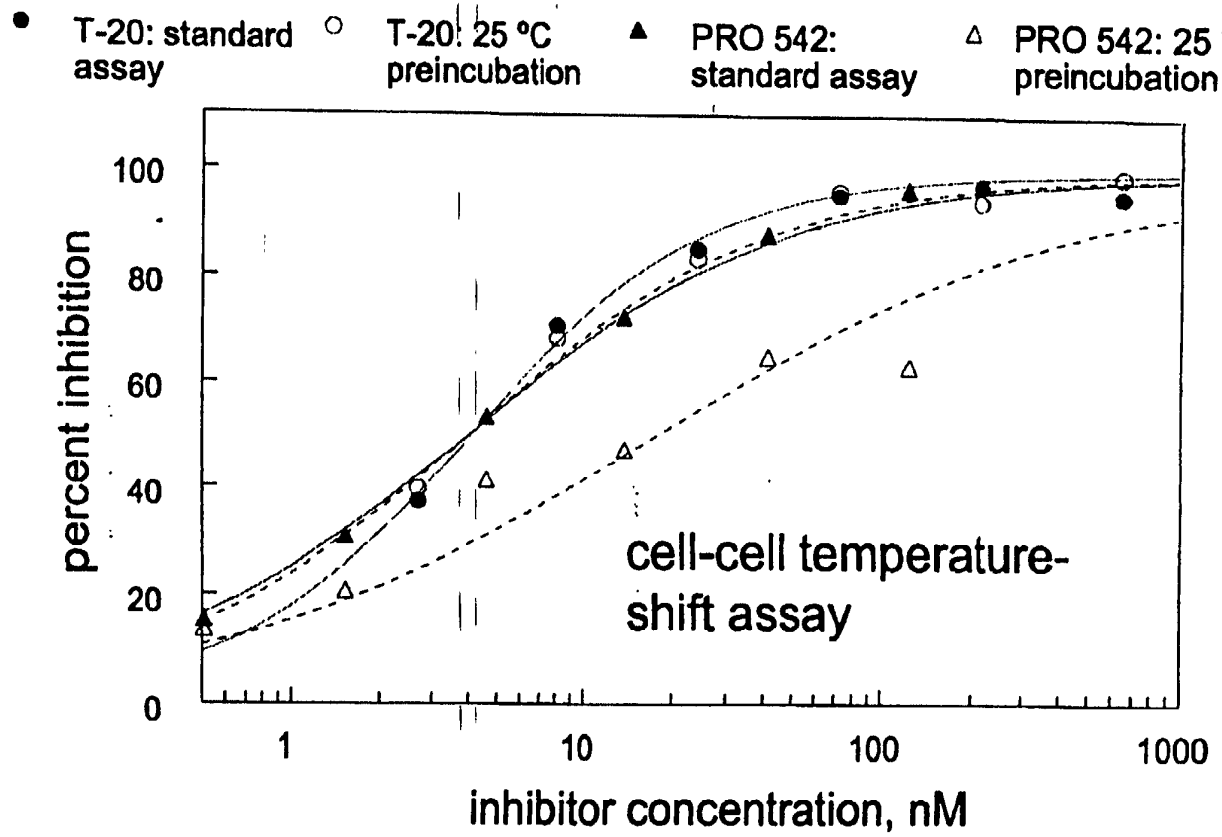

Figure 25

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (II)

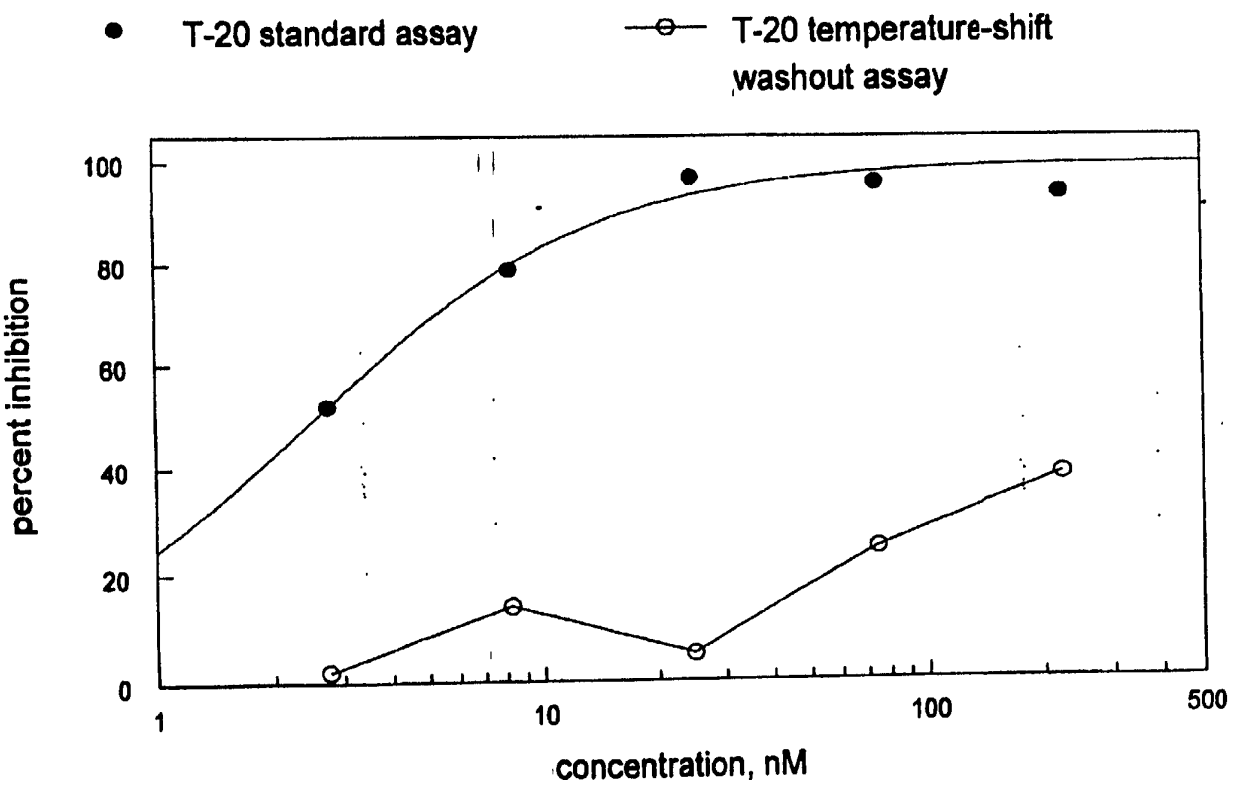

Figure 26

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

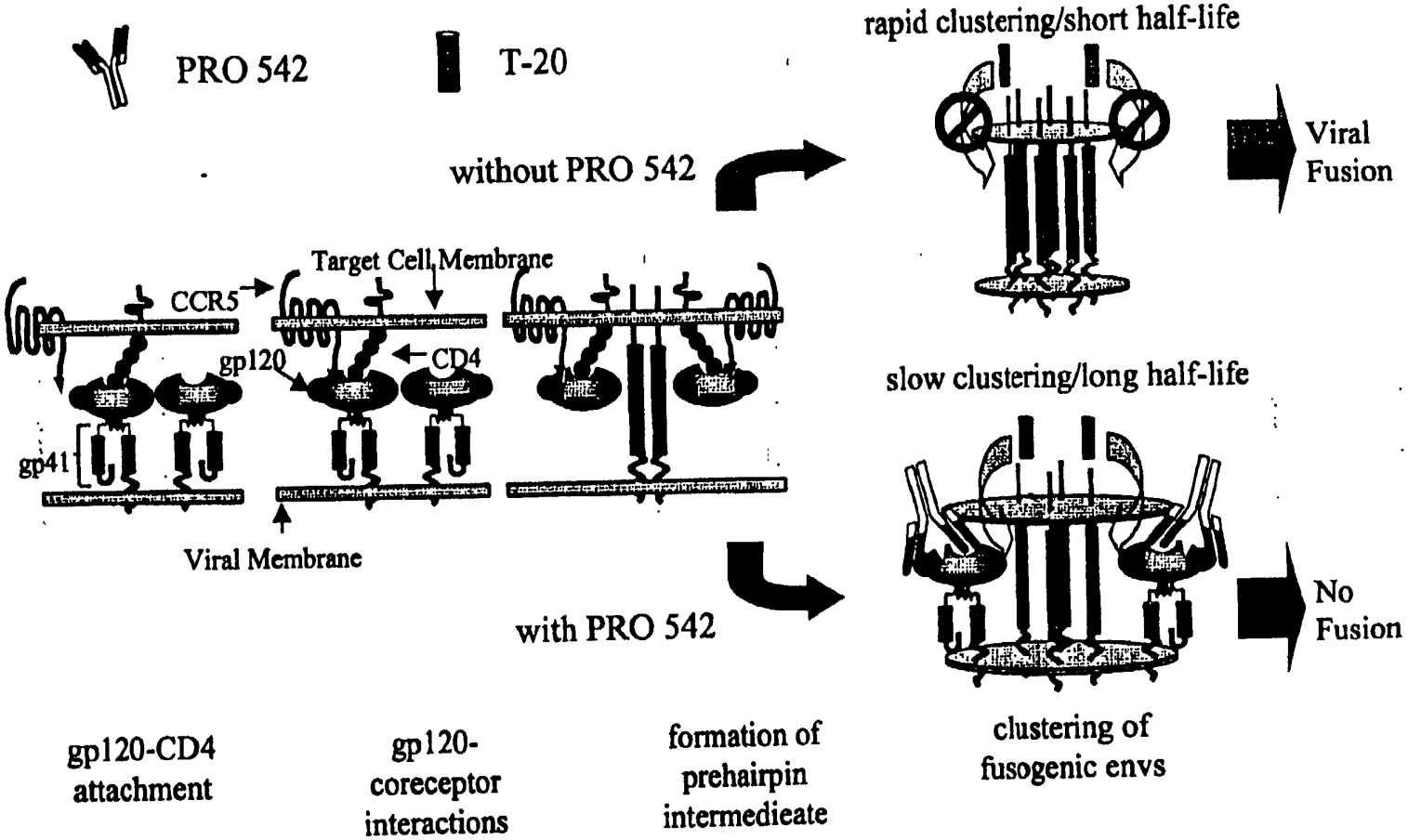

Figure 27

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

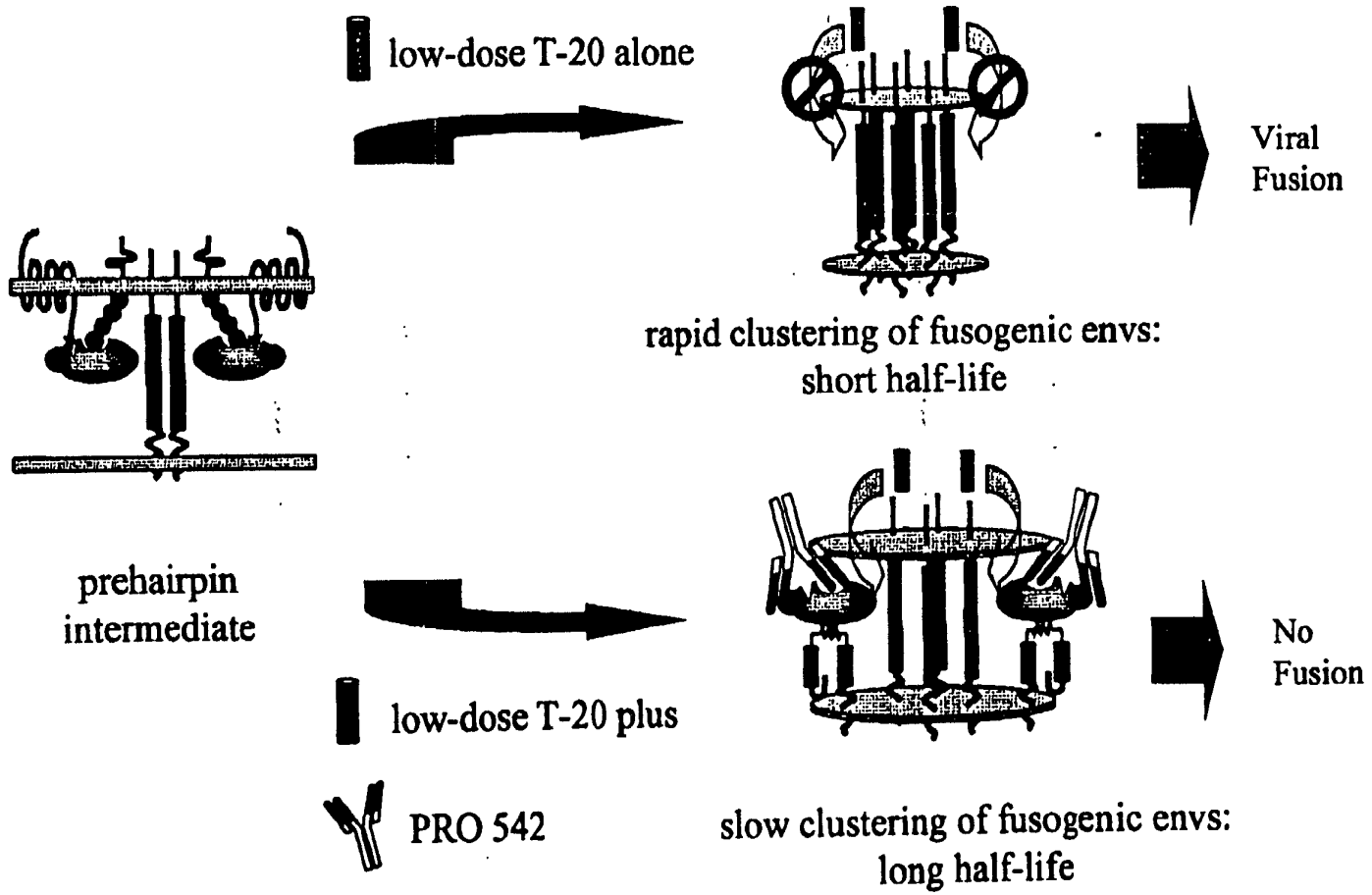

Figure 28

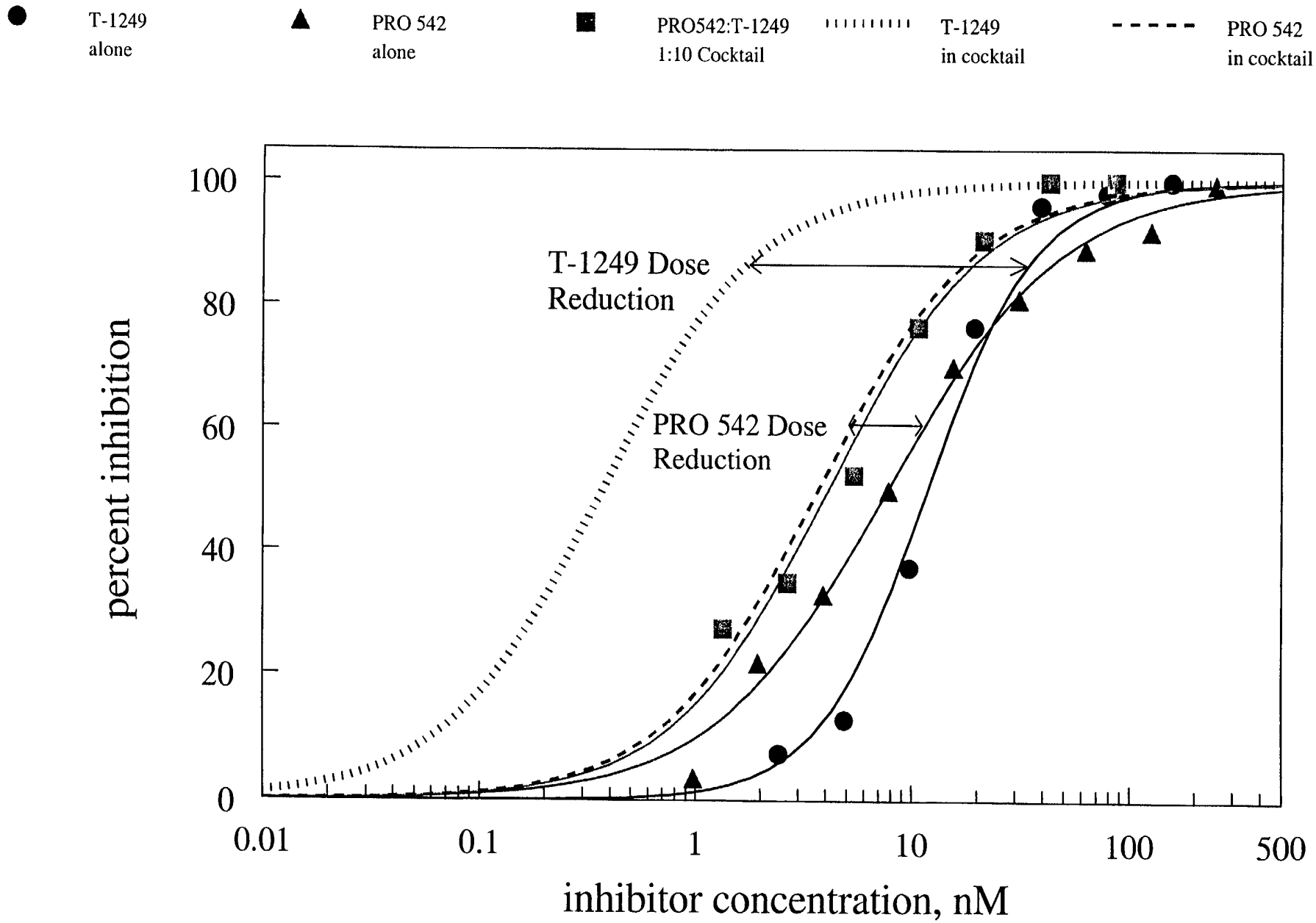
T-1249 alone
PRO 542 alone
PRO542:T-1249 1:10 Cocktail
T-1249 in cocktail
PRO 542 in cocktail
T-1249 Dose Reduction
PRO 542 Dose Reduction
percent inhibition
inhibitor concentration, nM
0
20
40
60
80
100
0.01
0.1
1
10
100
500

Figure 29

Fraction Inhibited	Dose PRO 542, nM (alone)	Dose PRO 542, nM (comb)	Dose T-1249, nM (alone)	Dose T-1249, nM (comb)	Combination Index	Dose Reduction PRO 542	Dose Reduction T-1249
0.95	87.90	13.58	37.83	1.36	0.20	6.47	27.86
0.90	48.69	9.52	27.11	0.95	0.24	5.12	28.48
0.85	33.78	7.64	22.06	0.76	0.27	4.42	28.87
0.80	25.65	6.47	18.88	0.65	0.30	3.96	29.17
0.75	20.43	5.65	16.61	0.56	0.32	3.62	29.42
0.70	16.75	5.01	14.85	0.50	0.34	3.34	29.64
0.65	13.99	4.50	13.41	0.45	0.37	3.11	29.84
0.60	11.81	4.06	12.20	0.41	0.39	2.91	30.03
0.55	10.05	3.68	11.13	0.37	0.41	2.73	30.21
0.50	8.57	3.35	10.18	0.33	0.44	2.56	30.39